# APPLICATION UNDER UNITED STATES PATENT LAWS

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Invention: INDOLY-3-GLYOXYLIC ACID DERIVATIVES HAVING THERAPEUTICALLY VALUABLE PROPERTIES

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This is a:

- ☐ Provisional Application
- ☐ Regular Utility Application
- ☒ Continuing Application
  - ☒ The contents of the parent are incorporated by reference
- ☐ PCT National Phase Application
- ☐ Design Application
- ☐ Reissue Application
- ☐ Plant Application
- ☐ Substitute Specification
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## SPECIFICATION

UNITED STATES PATENT AND TRADEMARK OFFICE

I, John William SPICER BSc, PhD, MRSC, CChem,

translator to RWS Group plc, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That I am well acquainted with the German and English languages.

3. That the attached is, to the best of my knowledge and belief, a true translation into the English language of the specification in German filed with the application for a patent in the U.S.A. on

under the number

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

J. W. Spicer

For and on behalf of RWS Group plc

The 22nd day of December 1999

**Indolyl-3-glyoxylic acid derivatives having therapeutically valuable properties**

The invention relates to the further advantageous embodiment of the German Patent Application indole-3-glyoxylamides having the reference 19814 838.0.

In connection with chemotherapy in the case of oncoses, the greatest problems result due to the occurrence of pharmaceutical resistance on the one hand and due to the serious side effects of these agents on the other hand.

In addition, it is known that after reaching a certain size many primary tumors prematurely tend to metastasis formation via the blood stream and lymphatic tracts. The progressive process of tumor invasion and the formation of metastases is the most frequent cause of death of the cancer patients.

There are various approaches for explaining this spread, inter alia enhanced angiogenesis, increased extracellular matrix degradation, tumor cell migration and modulation of cell adhesion. These factors can also interact but to date are only partially resolved.

The metastatic spread of a tumor is usually accompanied by poor prognoses in tumor treatment. The prerequisite for metastatic spread is the detachment of cells from the primary tumor, the migration of cells to the blood vessels, invasion into the blood vessels and invasion of the cells from the blood vessels into other tissue.

An inhibitory action of certain oncostatic agents such as tamoxoifen [sic] on the migration and invasion of cancer cells is known [J Clin Endocrinol Metab 1995 Jan.; 80(1): 308-13]

The inhibition of tumor cell invasion by verapamil has been reported [Pigment Cell Res 1991 Dec.; 4(5-6): 225-33.]

The influence of melantonin [sic] on invasive and metastatic properties of MCF-7 human breast cancer cells has been reported [Cancer res 1998 Oct. 1; 58(19): 4383-90]

In the published PCT Application WO 96/23506, the overcoming of pharmaceutical resistance in certain tumor pharmaceuticals was demonstrated as a result of the gene amplification of the multi-drug resistance gene (MDR gene) brought about by such oncostatic agents.

Oncostatic agents such as vincristine and Taxol furthermore have a not inconsiderable neurotoxicity, which proves disadvantageous in chemotherapy.

The object of the invention is then to widen the field of use of N-substituted indole-3-glyoxylamides and thus to enrich the available pharmaceutical wealth. The possibility of a lower, longer-lasting and better-tolerable medication for the class of substances having antitumor action described in German Patent Application 19814 838.0 should thus be opened up. In particular, the disadvantageous development of resistance, as is known of many antitumor agents, should be circumvented.

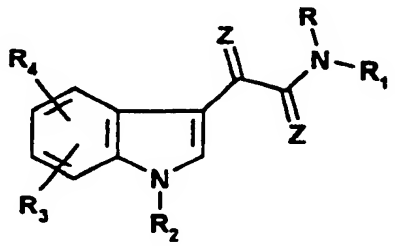
Moreover, development and spread of the tumor due to metastases should be counteracted.

According to more recent knowledge, as angiogenesis is obviously responsible for tumor growth and the development of metastases, the property of angiogenesis inhibition represents a further advantageous pharmaceutical potential, for example, in cancer therapy.

The increase in action achieved with the N-substituted indole-3-glyoxylamides should more effectively shape pharmaceutical consumption in tumor therapy. Moreover, it should be possible to shorten the period of treatment and to extend it in therapy-resistant cases. In addition, relapses and metastases should be restricted or prevented and thus the survival period of the patients additionally increased. The aim is to develop medicaments which can intervene in the process of metastatic spread.

It has surprisingly been found that the N-substituted indole-3-gloxylamides [sic] described in German Patent Application 19814 838.0, of the general formula 1 described below, which are suitable for the treatment of oncoses, further have those advantageous properties for tumor treatment which can extend their area of use.

The invention relates to the use of N-substituted indole-3-gloxylamides [sic] according to claim 1 general formula 1a for tumor treatment in particular in the case of pharmaceutical resistance and metastasizing carcinoma and for the suppression of metastasis formation, and also as angiogenesis inhibitors,




**Formula 1**

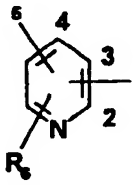
where the radicals R, $R_1$, $R_2$, $R_3$, $R_4$ and Z have the following meaning:

R = hydrogen, $(C_1-C_6)$-alkyl, where the alkyl group can be mono- or polysubstituted by the phenyl ring and

this phenyl ring for its part can be mono- or polysubstituted by halogen, $(C_1-C_6)$-alkyl, $(C_3-C_7)$-cycloalkyl, by carboxyl groups, carboxyl groups esterified with $C_1-C_6$-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups, benzyloxy groups and by a benzyl group which is mono- or polysubstituted in the phenyl moiety by $(C_1-C_6)$-alkyl groups, halogen atoms or trifluoromethyl groups,

R is further the benzyloxycarbonyl group (Z group) and the tertiary-butoxycarbonyl radical (BOC radical), furthermore the acetyl group.

$R_1$ can be the phenyl ring, which is mono- or polysubstituted by $(C_1-C_6)$-alkyl, $(C_1-C_6)$-alkoxy, cyano, halogen, trifluoromethyl, hydroxyl, benzyloxy, nitro, amino, $(C_1-C_6)$-alkylamino, $(C_1-C_6)$-alkoxycarbonylamino and by the carboxyl group or by the carboxyl group esterified with $C_1-C_6$-alkanols, or can be a pyridine structure of the formula 2 and its N-oxide [sic]




**Formula 2**

and its N-oxide, where the pyridine structure is alternatively bonded to the ring carbon atoms 2, 3 and 4 and can be substituted by the substituents $R_5$ and $R_6$. The radicals $R_5$ and $R_6$ can be identical or different and have the meaning $(C_1-C_6)$-alkyl and the meaning $(C_3-C_7)$-cycloalkyl, $(C_1-C_6)$-alkoxy, nitro, amino, hydroxyl, halogen and trifluoromethyl and further are the ethoxycarbonylamino radical and the group carboxyalkyloxy in which the alkyl group can have 1-4 C atoms.

$R_1$ can further be a 2- or 4-pyrimidinyl heterocycle, where the 2-pyrimidinyl ring can be mono- or polysubstituted by the methyl group, furthermore are [sic] the 2-, 3-, and 4- and 8-quinolyl structure substituted by $(C_1-C_6)$-alkyl, halogen, the nitro group, the amino group and the $(C_1-C_6)$-alkylamino radical, are [sic] a 2-, 3- and [sic] 4-quinolylmethyl group, where the ring carbons of the pyridylmethyl radical of the quinolyl group and of the quinolylmethyl radical can be substituted by $(C_1-C_6)$-alkyl, $(C_1-C_6)$-alkoxy, nitro, amino and $(C_1-C_6)$-alkoxycarbonylamino.

$R_1$ ,in the case in which R = hydrogen, the methyl or benzyl group and the benzyloxycarbonyl radical (Z radical), the tert-butoxycarbonyl radical (BOC radical) and the acetyl group, can furthermore be the following radicals:

$-CH_2COOH$; $-CH(CH_3)-COOH$; $-(CH_3)_2-CH-(CH_2)_2-CH-COO-$; $H_3C-H_2C-CH(CH_3)-$ $CH(COOH)-$[sic]; $HO-H_2C-CH(COOH)-$; phenyl-$CH_2-CH(COOH)-$; (4-imidazolyl)-$CH_2-CH-(COOH)-$; $HN=C(NH_2)-NH-(CH_2)_3-CH(COOH)-$; $H_2N-(CH_2)_4-CH(COOH)-$; $H_2N-CO-CH_2-CH-(COOH)-$; $HOOC-(CH_2)_2-CH(COOH)-$;

$R_1$ , in the case in which R is hydrogen, the Z group, the BOC radical, the acetyl or the benzyl group, can furthermore be the acid radical of a natural or unnatural amino acid, e.g. the α-glycyl, the α-sarcosyl, the α-alanyl, the α-leucyl, the α-isoleucyl, the α-seryl, the α-phenylalanyl, the α-histidyl, the α-prolyl, the α-arginyl, the α-lysyl, the α-asparagyl and the α-glutamyl radical, where the amino groups of the respective amino acids can be present unprotected or can be protected. A possible protective group of the amino function is the carbobenzoxy radical (Z radical) and the tert-butoxycarbonyl radical (BOC radical) as well as the acetyl group. In the case of the asparagyl and glutamyl radical claimed for

$R_1$, the second, unbonded carboxyl group is present as a free carboxyl group or in the form of an ester with $C_1$-$C_6$-alkanols, e.g. as a methyl, ethyl or as a tert-butyl ester.

Furthermore, $R_1$ can be the allylaminocarbonyl-2-methylprop-1-yl group.

R and $R_1$ can further form, together with the nitrogen atom to which they are bonded, a piperazine ring of the formula III or a homopiperazine ring, provided $R_1$ is an aminoalkylene group, in which

**Formula 3**

$R_7$ is an alkyl radical, is a phenyl ring which can be mono- or polysubstituted by ($C_1$-$C_6$)-alkyl, ($C_1$-$C_6$)-alkoxy, halogen, the nitro group, the amino function and by the ($C_1$-$C_6$)-alkylamino group. $R_7$ is furthermore the benzhydryl group and the bis-p-fluorobenzylhydryl [sic] group.

$R_2$ can be hydrogen and the ($C_1$-$C_6$)-alkyl group, where the alkyl group is mono- or polysubstituted by halogen and phenyl, which for its part can be mono- or polysubstituted by halogen, ($C_1$-$C_6$)-alkyl, ($C_3$-$C_7$)-cycloalkyl, carboxyl groups, carboxyl groups esterified with $C_1$-$C_6$-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups or benzyloxy groups. The ($C_1$-$C_6$)-alkyl group counting as $R_2$ can further be substituted by the 2-quinolyl group and the 2-, 3- and 4-pyridyl structure, which can both in each case be mono- or polysubstituted by halogen, ($C_1$-$C_4$)-alkyl groups or ($C_1$-$C_4$)-alkoxy groups. $R_2$ is further the aroyl radical, where the aryl moiety on which this radical is based is the phenyl ring, which can be mono- or polysubstituted by halogen, ($C_1$-$C_6$)-alkyl, ($C_3$-$C_7$)-cycloalkyl, carboxyl groups,

carboxyl groups esterified with $C_1$-$C_6$-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups or benzyloxy groups.

$R_3$ and $R_4$ can be identical or different and are hydrogen, ($C_1$-$C_6$)-alkyl, ($C_3$-$C_7$)-cycloalkyl, ($C_1$-$C_6$)-alkanoyl, ($C_1$-$C_6$)-alkoxy, halogen and benzyloxy. $R_3$ and $R_4$ can furthermore be the nitro group, the amino group, the ($C_1$-$C_4$)-mono or dialkyl-substituted amino group, and the ($C_1$-$C_6$)-alkoxycarbonylamino function or ($C_1$-$C_6$)-alkoxycarbonylamino-($C_1$-$C_6$)-alkyl function.

Z is O and S.

The designation alkyl, alkanol, alkoxy or alkylamino group for the radicals R, $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_7$ is normally understood as meaning both "straight-chain" and "branched" alkyl groups, where "straight-chain alkyl groups can be, for example, radicals such as methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl and "branched alkyl groups" designate, for example, radicals such as isopropyl or tert-butyl. "Cycloalkyl" is understood as meaning radicals such as, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl.
The designation "halogen" represents fluorine, chlorine, bromine or iodine. The designation "alkoxy group" represents radicals such as, for example, methoxy, ethoxy, propoxy, butoxy, isopropoxy, isobutoxy or pentoxy.

The compounds can also be employed as acid addition salts, for example as salts of mineral acids, such as, for example, hydrochloric acid, sulfuric acid, phosphoric acid, salts of organic acids, such as, for example, acetic acid, lactic acid, malonic acid, maleic acid, fumaric acid, gluconic acid, glucuronic acid, citric acid, embonic acid, methanesulfonic acid,

trifluoroacetic acid, succinic acid and 2-hydroxyethanesulfonic acid.

Both the compounds of the formula 1 and their salts are biologically active.

The compounds of the formula 1 can be administered in free form or as salts with physiologically tolerable acids.

Administration can be performed orally, parenterally, intravenously, transdermally or by inhalation.

The invention furthermore relates to pharmaceutical preparations which contain at least one of the compounds of the formula 1 or their salts with physiologically tolerable inorganic or organic acids and, if appropriate, pharmaceutically utilizable excipients and/or diluents or auxiliaries.

Suitable administration forms are, for example, tablets, coated tablets, capsules, solutions for infusion or ampoules, suppositories, patches, powder preparations which can be employed by inhalation, suspensions, creams and ointments.

The preparation processes for the substances can be taken from the examples of German Patent DE 196 36 150 A1.

The therapeutically valuable properties found relate specifically to the following advantages:

- no development of resistance was detected
- parameters were detected which are characteristic of the inhibition of metastasis formation (migration)
- parameters were found which confirm the inhibition of neovascularization (angiogenesis)

- in various models, it was not possible to find any neurotoxicity with the N-substituted indole-3-gloxylamides [sic] according to claim 1 general formula 1a in contrast to most antitumor preparations

The development of resistance which is not present is confirmed in the following pharmacological models and cell cultures:

1. The cytotoxic activity of D-24851 (see claim 4) on the MDR (multidrug-resistant) leukemia cell line of the mouse L 1210/VCR is not influenced in vivo and in vitro. See Figure 1, 2 and 3.

D-24851 (see claim 4) has an unchanged cytotoxic activity against the multidrug-resistant mouse leukemia cell subline L1210/VCR in contrast to Taxol, doxirubicin, vincristine or epotholone B [sic].

Experimental procedure:

The mouse leukemia cell lines [sic] L 120 was adapted to vincristine. The unadapted (L 1210) and the adapted (L 1210/VCR) cells were exposed to cytostatic agents and the cell growth, which was determined by the metabolic activity, was determined (XTT test).
The curves which connect the XTT datapoints were calculated using a nonlinear regression program.
These experimental results were also confirmed in vitro on the human resistant LT 12/MDR cell line see Figure 4.

2. The detection of lacking metastasis formation was afforded by means of inhibition of migration of MO4 cells. See Figure 5.
D-24 851 (see claim 4) inhibits the migration of MO4 cells in a dose-dependent manner. From this, an

antiinvasive and an antimetastatic action can be derived for D-24851.

The migration ability of MO4 cells can be measured in vitro by inoculating cells into the center of a cell culture dish and determining the migration by means of radius or the covered area of the cells after various days with and without D-24851. Figure 4 shows that the migration of the cells decreases with increasing D-24851 concentration.

In order to test whether D-24851 also acts antiinvasively, the invasion of MO4 fibrosarcoma cells into chickens' hearts was investigated. It is also seen here that at a concentration of 260 and 1000 nM the invasion is completely inhibited, whereas at lower concentrations the invasiveness of the MO4 cells increases. On the basis of these findings, it is seen that D-24851 inhibits both the migration and the invasion of tumor cells and thereby has a strong antimetastatic potential.

3. From comparison experiments of the compound according to the invention D-24851 (see claim 4) with vincristine and Taxol on rats, where ataxia, traction and reaction were assessed (see Figure 6), it is evident that this compound shows no neurotoxic effect, in contrast to Taxol and vincristine.

Furthermore, in comparison to Taxol and vincristine, D-24851 has no negative influence on the nerve conduction velocity see Figure 7.

This confirms that D-24851, on account of the absent neurotoxicity, has clearly lower side effects than other chemotherapeutics.

4. From further investigations as shown in Figure 8 and 9, it is evident that the compound D-25851 (see claim 4) has a potential as an angiogenesis inhibitor.

As a result of the physiological relationship with tumor growth, angiogenesis inhibitors are

simultaneously also agents for the inhibition of tumor growth, in that the formation of new blood vessels, which are intended to feed the tumor, is inhibited.

In vitro in an antiangiogenesis model on endothelial cells, D-24851 causes a complete inhibition of vascularization, which is not based on a cytotoxic effect.

It can be seen in Figure 8 that D-24851 almost completely breaks up existing cell-cell contacts due to 0.1 µMol/l of D 24851 [sic] (see vital staining). Normally, the cells maintain at least partial contact. Cell migration is markedly reduced, many cells are rounded.

Lethal staining in a monolayer before angiogenesis induction did not show any increased cell mortality with D-24851. Even in the first 22 hours after induction, no increased cell mortality was yet discernible in comparison with the control.

(See lethal staining in Figure 9, white points)

The cells originated from human umbilical vein (arterial function). They were employed for the investigation in the third and fourth passage. Angiogenesis is triggered by a natural stimulus. The primary trigger of endothelial migration is a protein which is expressed to an increased extent in vascularizing tissue. The substances are added to the culture medium shortly before induction of angiogenesis.

The concentration for the antiangiogenetic action of D-24851 is markedly below the concentration for the cytotoxic activity. As a result, it is possible to separate the two action qualities (cytotoxic activity and antiangiogenetic action) from one another.

Without wanting to restrict the scope of the invention by the following statements, it can be said that doses

from about 20 mg up to 500 mg daily are possible orally.

On intravenous administration as an injection or as an infusion, up to 250 mg/day or more can be administered depending on the body weight of the patient and individual tolerability.

As a result of the lacking development of resistance and suppression of metastasis, a high effectiveness and wide use of the agents for [sic] even in tumor-refractory patients can be expected.

The antiangiogenesis effect is suitable for additionally suppressing the spread of the tumor.

However, the invention also comprises the use of the N-substituted indole-3-gloxylamides [sic] according to claim 1 general formula 1a in further disorders in which an angiogenesis inhibitory effect is functionally desired. (e.g. wound healing)

In addition, the invention also relates to the fixed or free combination of the N-substituted indole-3-gloxylamides [sic] according to claim 1 general formula 1a with antitumor agents known per se, and also the replacement of antitumor agents which have become ineffective as a result of resistance development by N-substituted indole-3-gloxylamides [sic] according to claim 1 general formula 1a.